

## Fungal ene-reductases as sustainable synthetic tools for the reduction of chemical compounds.

Alice Romagnolo<sup>1</sup>, Federica Spina<sup>1</sup>, Michele Crotti<sup>2</sup>, Fabio Parmeggiani<sup>2</sup>, Elisabetta Brenna<sup>2</sup>, Giovanna Cristina Varese<sup>1</sup>

<sup>1</sup> Department of Life Science and Systems Biology, University of Turin, viale Pier Andrea Mattioli 25, 10125 Turin, Italy

<sup>2</sup> Department of Chemistry, Materials and Chemical Engineering, Politecnico di Milano, via Luigi Mancinelli 7, 20131 Milan, Italy

Bio-based technologies are useful to reduce the high costs and environmental impact of traditional chemical processes. Biocatalysis is particularly attractive for the low energy demand, waste and by-products formations and the reduced costs. Moreover this method allows to produce pure enantiomers highly valued in pharmaceutical and flavour fields. Microorganisms and their enzymatic derivatives are interesting for the production of fine chemicals, pharmaceuticals and agrochemical intermediates. Fungi are worthy biocatalysts due to the natural biodiversity and the broad heterogeneous enzymatic pattern. The reduction of C=C double bonds is widely used in organic chemistry; currently it is performed by highly polluting and expensive metal catalysts. A viable alternative may be the use of ene-reductases which perform the reduction of C=C double bonds conjugated with different electron-withdrawing groups such as carbonyl, nitro and ester. To date, these enzymes are poorly investigated in filamentous fungi.

This research aims to develop fungal whole-cell catalysed processes to provide new sustainable synthetic tools for organic chemistry, producing useful molecules such as enantiopure chiral compounds. This study focused on the investigation of ene-reductase activities in different species of filamentous fungi belonging to Basidiomycota, Ascomycota and Zygomycota, analysed in the bioconversion of precursors of industrial compounds.

Twenty-eight filamentous fungi were analysed in the conversion of cyclohexenone,  $\alpha$ -methylnitrostyrene,  $\alpha$ -methylcinnamaldehyde and methyl cinnamate. Almost all the fungi showed ene-reductase activities and transformed the substrates with quite high conversion rate producing molecules which can be used for instance in the flavours production (i.e., methyl dihydrocinnamyl alcohol from  $\alpha$ -methylcinnamaldehyde). In particular *Gliomastix maseei*, *Mucor circinelloides* and *Mucor plumbeus* resulted versatile and effective reducing all the substrates quickly and with high yields.